Chapter 8

Tacrolimus pharmacokinetics and pharmacogenetics: influence of adenosine triphosphate-binding cassette B1 (ABCB1) and cytochrome (CYP) 3A polymorphisms

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Abstract

Background and aim

Tacrolimus, an immunosuppressant used after organ transplantation, has a narrow therapeutic range and its pharmacokinetic variability complicates its daily dose assessment. P-glycoprotein (P-gp), encoded by the adenosine triphosphate-binding cassette B1 gene (ABCB1) and the cytochrome (CYP) 3A4 and 3A5 enzymes appear to play a role in the tacrolimus metabolism.

Materials and methods

In the present study, two different renal transplant recipients groups are used to examine the influence of ABCB1 and CYP3A polymorphisms on the daily tacrolimus dose and several pharmacokinetic parameters. In total 63 Caucasian renal transplant recipients divided into 26 early and 37 late posttransplant recipients were genotyped for ABCB1 and CYP3A polymorphisms. The pharmacokinetic parameters of tacrolimus were determined for all renal transplant recipients and correlated with their corresponding genotypes.

Results

A significant difference in allele frequencies of the CYP3A4*1B (P = 0.028) and CYP3A5*1 (P = 0.022) alleles was observed between the early and late posttransplant recipient group. Significant higher dose-normalised trough levels (dnC_0), dose-normalised area under the curve ($dnAUC_{0\text{-}12}$), and dose-normalised maximum concentration (dnC_{max}) were observed for carriers of the CYP3A5*3 variant allele in the two renal transplant patient groups. Except for the daily tacrolimus dose (P = 0.025) no significant differences were observed for carriers of the CYP3A4*1B variant allele. Neither the individual ABCB1 polymorphisms nor the ABCB1 haplotypes were associated with any pharmacokinetic parameter.

Conclusion

We noticed that a complete pharmacokinetic profile is more frequently requested for renal transplant recipients carrying a CYP3A5*1 allele suggesting that these patients have more difficulties in achieving and maintaining tacrolimus concentrations compared to homozygous carriers of the CYP3A5*3 variant allele. Additionally, patients carrying a CYP3A5*1 allele require a twofold higher tacrolimus dose compared to homozygous carriers of the CYP3A5*3 variant allele to maintain the target $dnAUC_{0\text{-}12}$. Therefore, genotyping for the CYP3A5*3 variant allele can contribute to a better and more individualized immunosuppressive therapy in transplant patients.

Introduction

The immunosuppressant tacrolimus is used worldwide in transplant patients although its narrow therapeutic window and high pharmacokinetic variability complicate the establishment of a dosage regime[1,2]. Therefore, close therapeutic drug monitoring for tacrolimus is required to prevent the risk of subtherapeutic or toxic blood concentrations. Subtherapeutic tacrolimus blood concentrations increase the risk of transplant rejection enormously[3-5], while high tacrolimus blood concentrations may lead to severe side effects such as nephrotoxicity, neurotoxicity and hyperglycemia[6-8]. Tacrolimus is like numerous other clinically used drugs, a substrate for P-glycoprotein (P-gp) and cytochrome P450 3A (CYP3A) enzymes. P-gp is encoded by the adenosine triphosphate (ATP)-binding cassette B1 (ABCB1) gene and co-expressed with the CYP3A enzymes in the liver and intestines. CYP3A4 and CYP3A5 are the most important members of the CYP3A subfamily[9,10]. The differences in expression levels and activity of ABCB1 and CYP3A may explain the inter-individual variations in the tacrolimus pharmacokinetics. P-gp is an ATP-dependent membranous transporter that contributes to the protection of the body from environmental toxins and drugs like tacrolimus by limiting their absorption from the intestine and promoting the efflux into bile and urine[11]. Three partly linked polymorphisms in the ABCB1 gene located on exons 12, 21 and 26 have been studied extensively. Two of these ABCB1 polymorphisms, C1236T and C3435T, result in silent mutations while the ABCB1 G2677T/A polymorphism in exon 21 is non-synonymous and results in an amino acid exchange (Ala893Ser/Thr). A number of studies found no association between the ABCB1 genotypes and tacrolimus trough (C_0) concentrations[12-17] or the pharmacokinetic parameters: area under the time tacrolimus concentration curve (AUC_{0-12}), the maximum concentration (C_{max}), the time that the maximum concentration is reached (T_{max}) and the half-life time ($t_{1/2}$)[18,19]. However, some other studies found an association between the tacrolimus C_0 concentrations, the daily tacrolimus dose and individual ABCB1 polymorphisms[20,21] or the ABCB1 haplotypes[22]. Homozygotes for the CYP3A4*1B and CYP3A5*3 variant allele carry at position –392 an A and at position 6986 a G, respectively. Moreover, homozygotes for the CYP3A4*1B variant allele show an altered CYP3A4 enzyme activity while homozygotes for the CYP3A5*3 variant allele show no CYP3A5 enzyme activity. A number of studies already demonstrated the impact of the CYP3A5*3 variant allele on tacrolimus C_0 concentrations[12-17,23-25], the pharmacokinetic parameters: AUC_{0-12}, C_{max}, T_{max}, $t_{1/2}$[18,19] and the daily tacrolimus dose in different transplant patient groups. However, the influence of CYP3A4*1B and ABCB1 C1236T variant alleles on these pharmacokinetic parameters of tacrolimus has never been examined. In the present study, a complete 12 hour pharmacokinetic profile is recorded for all transplant patients and the influence of several clinical and genetic parameters is determined on the variation in pharmacokinetic parameters of tacrolimus in an early and a late posttransplant recipient group. Additionally, the association will be examined between the CYP3A4 A-392G, CYP3A5 A6986G, CYP3AP1 G-44A

polymorphisms and the three ABCB1 polymorphisms C1236T, G2677T/A, C3435T on the pharmacokinetic parameters in the same renal transplant recipient groups.

Patients and methods

Study population

In total 63 Caucasian renal transplant recipients of whom a complete 12 hour time tacrolimus concentration curve was performed were divided over two different groups. Table 8.1 illustrates that most pharmacokinetic profiles of the patients included in group I were recorded within 6 weeks after transplantation, while group II included patients that underwent a renal transplantation at least 1 year ago. Moreover, in group I eight patients used calcium channel blockers which are known to interact with tacrolimus, whereas patients included in group II used no medication known to interfere with tacrolimus. In addition, patients that suffer from gastrointestinal, liver disease or other disorders that may alter the absorption of tacrolimus were disqualified for inclusion in both groups. Prior to the blood sample collection, there had been no tacrolimus dose change for at least three days in the two groups. After overnight fasting the blood samples were collected immediately pre (C_0) and 0.5 ($C_{0.5}$), 1 (C_1), 2 (C_2), 3 (C_3), 4 (C_4), 5 (C_5), 7.5 ($C_{7.5}$) and 12 (C_{12}) hours after the morning tacrolimus administration. Patients were not allowed to take food until 1 hour after ingesting the tacrolimus dose and were advised to avoid grapefruit juice after transplantation to prevent alterations in the tacrolimus metabolism. Demographic as well as clinical data were determined at the time of recording the 12 hour time tacrolimus concentration curve.

Ethics

The study was performed in accordance to the Declaration of Helsinki and its amendments. The protocol was approved by the local Medical Ethics Committee and written informed consent for participation in this study was obtained from all patients.

Tacrolimus concentration determinations

The tacrolimus blood concentrations were determined in ethylene diamine tetra-acetic acid (EDTA) whole blood, using a microparticle enzyme immunoassay with a monoclonal antibody (IMx II assay; Abbott Laboratories, Abbott Park, IL, USA) for group I and a method based on high pressure liquid chromotography (LC) tandem mass spectrometry (MS/MS) for group II. The laboratories in which the tacrolimus concentrations have been determined participate in the International Tacrolimus Proficiency Testing Scheme.

Table 8.1 Demographic characteristics of the two renal transplant recipients groups.

Demographic characteristics	Group I	Group II
Gender (male/female)	18/8	24/13
Age (years, mean ± SD)	43.0 ± 13.2	51.3 ± 10.9
Body Mass Index (kg/m^2, mean ± SD)	23.3 ± 4.40	25.6 ± 3.42
Primary kidney disease		
Glomerulonephritis	4	1
Chronic pyelonephritis	2	2
IgA nephropathy	3	4
Hypertensive nephropathy	4	7
Diabetes Mellitus nephropathy	4	0
Polycystic kidney disease	1	8
Unknown	1	4
Other	7	11
Transplantation number		
First	20	30
Second	5	6
Third or more	1	1
Tacrolimus mono therapy	1	29
Tacrolimus dose (mg/kg/day, mean ± SD)	0.39 ± 0.231	0.054 ± 0.029
C_0 (ng/ml, mean ± SD)	16.8 ± 5.83	6.59 ± 1.39
$AUC_{0\text{-}12}$ (ng × hr/ml, mean ± SD)	305.0 ± 96.8	122.5 ± 31.1
C_{max} (ng/ml, mean ± SD)	56.3 ± 21.3	20.9 ± 6.5
T_{max} (hr, mean ± SD)	1.46 ± 1.33	1.24 ± 0.43
Use of azothiopurine, MMF[a], rapamycine, steroids	11/3/4/26	3/4/0/0
Current steroid dose (mg, dose, no. patients)		
0 mg/day	3	37
5 mg/day	2	0
8 mg/day	2	0
10 mg/day	10	0
15 mg/day	4	0
20 mg/day	3	0
> 20 mg/day	2	0
Time since transplantation (days, median, (range))	16 (3-74)	1465 (453–4128)
Haemoglobin (mmol/l, mean ± SD; ref. ♂ 8.2-11.0, ♀ 7.3-9.7)	5.43 ± 1.08	8.52 ± 0.83
Haematocrit (mean ± SD; ref. ♂ 0.41-0.52, ♀ 0.36-0.48)	0.25 ± 0.07	0.41 ± 0.04
ALAT (U/l, mean ± SD; ref. ♂ < 45, ♀ < 35)	34 ± 34	24 ± 13
Serum albumin (g/l, mean ± SD; ref. 34-45)	30.7 ± 3.86	37.0 ± 3.84
Serum creatinine (µmol/l, mean ± SD; ref. ♂ 71-110, ♀ 53-97)	331 ± 293	128 ± 29
Creatinine clearance[b] (ml/min, mean ± SD; ref. 90-140)	37.5 ± 28.4	58.0 ± 26.6

ref. are the reference values applied in the clinical chemistry and haematology laboratory of the University Hospital Maastricht. ♂ male, ♀ female, [a] MMF is mycophenolate mofetil, [b] The creatinine clearance is determined with the Cockcroft-Gault equation.

The tacrolimus C_0 concentration and the peak tacrolimus blood concentration (C_{max}) during the assessed time interval were determined directly from the time *versus* tacrolimus blood concentration data. Additionally, the area under the time tacrolimus concentration curve ($AUC_{0\text{-}12}$) was calculated from the time *versus* tacrolimus concentration plot using the linear trapezoidal rule in MWPharm 3.50 (Mediware, Groningen, the Netherlands). Dose-normalised (Dn)C_0, dose-normalised (dn)$AUC_{0\text{-}12}$

and dose-normalised (dn)C_{max} were calculated by dividing the C_0, AUC_{0-12} and C_{max}, respectively, by the corresponding morning dose on a milligram per kilogram basis.

DNA isolation

Genomic DNA isolation was performed on 63 renal transplant recipients by using 200 µl EDTA anticoagulated blood for isolation with a QIAamp blood mini kit (Qiagen, Leusden, the Netherlands) according to the manufacturers' instructions.

Genotyping of ABCB1 and CYP3A gene polymorphisms

Real-time polymerase chain reaction (PCR) fluorescence resonance energy transfer (FRET) assays were used for genotyping, ABCB1 G2677T/A, ABCB1 C3435T, CYP3A4 A-392G and CYP3AP1 G-44A using the same primers and probes compared to the original publications[26-29]. Regarding the ABCB1 C1236T and CYP3A5 A6986G polymorphisms real-time PCR FRET assays were used as we described earlier[30]. For each polymorphism, heterozygote samples were sequenced according to a direct sequence procedure on a capillary sequencer ABI Prism 3100 using the Bridge version 1.1 sequence kit (both products from Applied Biosystems, Fostercity, USA) and used in every real-time PCR FRET assay run as control sample.

Statistical analysis

Statistical analysis of the data was performed with use of the statistical software SPSS 11.0 for windows (Chicago, IL, USA). Correlations between pharmacokinetic parameters and clinical variables were evaluated using stepwise multiple regression analysis. To examine the population homogeneity of the patients, the genotype frequencies of the ABCB1 and CYP3A polymorphisms were tested against Hardy-Weinberg equilibrium by the Pearson's goodness-of-fit test[31]. Allele frequencies of the early and late posttransplant recipient group were compared using the χ^2 test. For analysis of the daily tacrolimus dose (mg/kg/day), dnC_0 (ng/ml per mg/kg), $dnAUC_{0-12}$ (ng × hr/ml per mg/kg), and dnC_{max} (ng/ml per mg/kg), groups were compared using non parametric statistical tests. To compare two groups we used the Mann-Whitney test, and to compare several groups the Kruskal Wallis test. *P* values less than 0.05 were considered statistically significant. All values are expressed as median and range unless stated otherwise.

Results

Influence of clinical and genetic parameters on the variation in pharmacokinetic tacrolimus parameters

The characteristics of the early posttransplant (group I) as well as the late posttransplant (group II) recipients that were enrolled in our study are shown in Table 8.1.

Tabel 8.2 Influence of independent clinical and genetic parameters on the pharmacokinetic parameters of an early (group I) and a late (group II) posttransplant recipient group.

Group	Dependent parameter	Independent parameter	Partial r^2	^aP value
I	Tacrolimus dose	CYP3A5 A6986G polymorphism	0.531	< 0.001
		Model r^2: 0.531		
I	DnC_0	Time since transplantation	0.613	< 0.001
		Haemoglobin	0.079	< 0.001
		Model r^2: 0.692		
I	$DnAUC_{0\text{-}12}$	Time since transplantation	0.528	< 0.001
		Haemoglobin	0.092	< 0.001
		Model r^2: 0.620		
I	DnC_{max}	Time since transplantation	0.405	< 0.001
		Haemoglobin	0.230	< 0.001
		Model r^2: 0.635		
II	Tacrolimus dose	CYP3A5 A6986G polymorphism	0.354	< 0.001
		Model r^2: 0.354		
II	DnC_0	CYP3A5 A6986G polymorphism	0.175	0.010
		Body mass index (BMI)	0.157	0.001
		Gender	0.086	< 0.001
		Model r^2: 0.418		
II	$DnAUC_{0\text{-}12}$	CYP3A5 A6986G polymorphism	0.190	0.007
		Body mass index (BMI)	0.145	0.001
		Model r^2: 0.335		
II	DnC_{max}	CYP3A5 A6986G polymorphism	0.226	0.003
		Body mass index (BMI)	0.109	0.001
		Serum Albumin	0.077	< 0.001
		Model r^2: 0.412		

^aP value belonging to the partial r^2. DnC_0, dose-normalised trough level, $dnAUC_{0\text{-}12}$, dose-normalised area under the curve, DnC_{max} dose-normalised maximum concentration. Tested independent parameters were gender, age, body mass index (BMI), haemoglobin, haematocrit, ALAT, serum albumin, serum creatinine, creatinine clearance (Cockcroft-Gault), CYP3A4 A-392G, CYP3A5 A6986G, ABCB1 C1236T, G2677T/A, C3435T.

The influence of different clinical (gender, age, body mass index (BMI), haemoglobin, haematocrit, ALAT, serum albumin, serum creatinine, creatinine clearance) and genetic (CYP3A4 A-392G, CYP3A5 A6986G, ABCB1 C1236T, G2677T/A, C3435T) parameters is examined on the variation in the tacrolimus pharmacokinetic parameters in all posttransplant recipients. Table 8.2 illustrates the influence of independent clinical and genetic parameters on the variability of pharmacokinetic tacrolimus parameters. In the

early posttransplant recipient group the time since transplantation and the haemoglobin concentration correlate significantly with dnC_0, $dnAUC_{0-12}$ and dnC_{max} whereas the CYP3A5 A6986G polymorphism and the body mass index (BMI) have a significant contribution on the variability of dnC_0, $dnAUC_{0-12}$ and dnC_{max} in the late posttransplant recipient group. The clinical and genetic parameters: age, haematocrit, ALAT, serum creatinine, creatinine clearance and the polymorphisms CYP3A4 A-392G, ABCB1 C1236T, ABCB1 G2677T/A and ABCB1 C3435T show no significant correlation with any of the pharmacokinetic parameters in both renal transplant recipient groups.

Allele distribution of the different ABCB1 and CYP3A polymorphisms

Table 8.3 and 8.4 show the genotype frequencies of the different CYP3A and CYP3AP1 polymorphisms that were determined for the 26 early and 37 late posttransplant recipients. The genotype frequencies of the two renal transplant recipient groups were not significantly different from that predicted by the Hardy-Weinberg equation.

Table 8.3 Influence of ABCB1, CYP3A and CYP3AP1 allelic variants on the pharmacokinetic tacrolimus parameters of the early posttransplant recipients.

Genotype	N No	Allelic status	Dose	DnC_0	$DnAUC_{0-12}$	DnC_{max}
CYP3A4	21	*1A/*1A	0.28 (0.04-0.80)[a]	90 (40-1329)	1782 (624-15721)	383 (89-1535)
A-392G	5	*1A/*1B	0.50 (0.43-0.79)[a]	59 (32-107)	1275 (922-1796)	216 (184-338)
	0	*1B/*1B	-----	-----	-----	-----
CYP3A5	1	*1/*1	0.78	32	922	184
A6986G	9	*1/*3	0.50 (0.35-0.80)[a]	59 (40-107)[a]	1220 (824-1796)[a]	216 (89-380)[a]
	16	*3/*3	0.25 (0.04-0.59)[a]	118 (45-1329)[a]	1975 (839-15721)[a]	435(90-1535)[a]
CYP3AP1	1	G/G	0.78	32	922	184
G-44A	10	G/A	0.49 (0.12-0.80)[a]	60 (40-107)[a]	1248 (824-1796)[a]	236 (89-381)[a]
	15	A/A	0.25 (0.04-0.59)[a]	123 (45-1329)[a]	2030 (839-15721)[a]	454 (90-1535)[a]

Tacrolimus dose (mg/kg/day), dnC_0, dose-normalized trough concentration (ng/ml per mg/kg), $dnAUC_{0-12}$ (ng × hr/ml per mg/kg) dose-normalised area under the curve, dnC_{max} dose-normalised maximum concentration (ng/ml per mg/kg). Values are indicated as median and (range), [a] $P < 0.05$, (Mann-Whitney).

Effect of ABCB1 and CYP3A polymorphisms on the daily tacrolimus dose and the pharmacokinetic parameters

As is demonstrated in Table 8.5 and Table 8.6, we found that neither the individual ABCB1 polymorphisms nor the ABCB1 haplotypes are associated with the daily tacrolimus dose and the pharmacokinetic parameters; dnC_0, $dnAUC_{0-12}$ and dnC_{max}. Table 8.3 shows a trend between the heterozygote carriers of the CYP3A4*1B variant allele in both the daily tacrolimus dose and the pharmacokinetic tacrolimus parameters compared to the homozygote carriers of the CYP3A4*1A allele. However, except for the daily tacrolimus dose (0.28 *versus* 0.50 mg/kg/day; Mann-Whitney, $P = 0.025$) no

significant differences are found between the pharmacokinetic parameters for tacrolimus and the different CYP3A4 A-392G genotypes in the early posttransplant recipient group.

Table 8.4 Influence of ABCB1, CYP3A and CYP3AP1 allelic variants on the pharmacokinetic tacrolimus parameters of the late posttransplant recipients.

Genotype	N No	Allelic status	Dose	DnC_0	$DnAUC_{0\text{-}12}$	DnC_{max}
CYP3A4	36	*1A/*1A	0.05 (0.02-0.14)	276 (70-669)	4642 (1355-11994)	775 (294-1729)
A-392G	1	*1A/*1B	0.067	121	2766	504
	0	*1B/*1B	-----	-----	-----	-----
CYP3A5	0	*1/*1	-----	-----	-----	-----
A6986G	5	*1/*3	0.10 (0.05-0.14)[a]	87.4 (70-248)[a]	1663 (1355-4057)[a]	461 (294-646)[a]
	32	*3/*3	0.04 (0.02-0.11)[a]	287 (118-669)[a]	4808 (2227-11994)[a]	814 (465-1729)[a]
CYP3AP1	0	G/G	-----	-----	-----	-----
G-44A	8	G/A	0.07 (0.02-0.14)[a]	161 (70-669)[a]	2990 (1355-11994)[a]	544 (294-1729)[a]
	29	A/A	0.04 (0.02-0.11)[a]	283 (118-640)[a]	4761 (2227-11504)[a]	810 (465-1493)[a]

Tacrolimus dose (mg/kg/day), dnC_0, dose-normalised trough concentration (ng/ml per mg/kg), $dnAUC_{0\text{-}12}$ (ng×hr/ml per mg/kg) dose-normalized area under the curve, dnC_{max} dose-normalised maximum concentration (ng/ml per mg/kg). Values are indicated as median and (range), [a] $P < 0.05$; (Mann-Whitney).

Additionally, Table 8.3 demonstrates a significant decrease in the pharmacokinetic tacrolimus parameters dnC_0, 118 *versus* 59 ng/ml per mg/kg; $dnAUC_{0\text{-}12}$ 1975 *versus* 1220 ng × hr/ml per mg/kg; and dnC_{max} 435 *versus* 216 ng/ml per mg/kg when renal transplant recipients were carrier of none or one CYP3A5*1 allele, respectively. Consequently, the daily tacrolimus dose is significantly higher in heterozygous carriers of the CYP3A5*3 variant allele compared to homozygous carriers of the CYP3A5*3 variant allele (0.50 *versus* 0.25 mg/kg/day; Mann-Whitney, $P = 0.001$). Regarding the late posttransplant recipients included in group II, a similar genetic effect is found for CYP3A5*1 allele in association with the pharmacokinetic tacrolimus parameters and the daily tacrolimus dose, as is shown in Table 8.4. Because there is only one heterozygous carrier of the CYP3A4*1B variant allele among the late posttransplant group, no statistical analyses could be performed between the CYP3A4*1B genotypes and the pharmacokinetic tacrolimus parameters. Although we observed a significant difference in the daily tacrolimus dose as well as in the dnC_0, $dnAUC_{0\text{-}12}$ and dnC_{max} for only the CYP3A5*3 variant allele, it is not clear whether the CYP3A4*1B variant allele has, similarly as the CYP3A5*3 variant allele, an important contribution to the pharmacokinetic variability of tacrolimus. Particularly, knowing that in this study all individuals carrying a CYP3A4*1B variant allele also carry at least one CYP3A5*1 allele. To examine the influence of the CYP3A4*1B variant allele solely, we selected those renal transplant recipients in group I that were heterozygous for the CYP3A5*1 allele. One renal transplant patient was heterozygote for the CYP3A4*1B variant allele and homozygote for the CYP3A5*1 allele. Five renal transplant patients were carrier of both one CYP3A4*1B variant allele and one CYP3A5*1 allele. Additionally, another four renal

transplant recipients were homozygous for the CYP3A4*1A allele and heterozygous for one CYP3A5*1 allele. Figure 8.1 illustrates renal transplant recipients who are heterozygous for the CYP3A4*1B and CYP3A5*3 variant allele show no significantly higher daily tacrolimus dose or lower $dnAUC_{0\text{-}12}$ compared to patients that were only heterozygous for the CYP3A4*1B variant allele.

Table 8.5 ABCB1 haplotypes related to tacrolimus dose requirement and dose-normalised $AUC_{0\text{-}12}$ of the early posttransplant recipients.

ABCB1 haplotypes			N	Dose	$DnAUC_{0\text{-}12}$
C1236T	G2677T/A	C3435T	No	(mg/kg/day)	(ng × hr/ml per mg/kg)
CC	GG	CC	3	0.78 (0.23-0.79)	1333 (922-1737)
CC	GG	CT	3	0.23 (0.04-0.49)	2030 (1275-6186)
CC	GA	CC	2	0.34 (0.04-0.65)	8292 (864-15721)
CT	GT	CC	1	0.46	1097
CT	GT	CT	6	0.30 (0.22-0.59)	1570 (1103-3032)
CT	GT	TT	3	0.28 (0.25-0.42)	1920 (1821-2066)
CT	GG	CC	1	0.54	839
CT	GG	TT	1	0.35	1321
TT	TT	TT	6	0.40 (0.07-0.80)	1789 (824-8964)

Values are indicated as median and (range).

Table 8.6 ABCB1 haplotypes related to tacrolimus dose requirement and dose-normalised $AUC_{0\text{-}12}$ of the late posttransplant recipients

ABCB1 haplotypes			N	Dose	$DnAUC_{0\text{-}12}$
C1236T	G2677T/A	C3435T	No	(mg/kg/day)	(ng × hr/ml per mg/kg)
CC	GG	CC	3	0.03 (0.02-0.04)	8798 (5010-9320)
CC	GG	CT	7	0.06 (0.03-0.08)	4057 (2227-5216)
CC	GG	TT	3	0.04 (0.02-0.06)	4465 (3147-6854)
CC	GA	CC	1	0.07	3215
CT	GT	CC	1	0.07	3081
CT	GT	CT	13	0.05 (0.02-0.13)	4761 (1355-9520)
CT	GT	TT	2	0.09 (0.04-0.14)	4571 (1662-7481)
TT	GT	CT	1	0.05	4511
TT	TT	CT	1	0.02	11504
TT	TT	TT	5	0.04 (0.02-0.07)	4718 (2766-11994)

Values are indicated as median and (range).

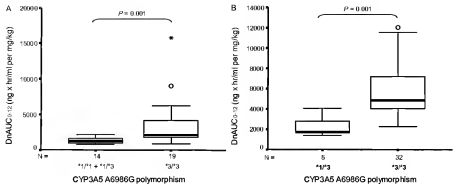

Figure 8.1 Boxplot of the distribution of (A) the dose-normalised (Dn)$AUC_{0\text{-}12}$ (ng × hr/ml per mg/kg) and (B) the tacrolimus dose (mg/kg/day) clustered according to the combination of CYP3A4 A-392G and CYP3A5 A6986G genotypes. *P* values are given for the pairwise comparisons of each genotype combination.

Discussion

In the early posttransplant recipient group the time since transplantation and the haemoglobin concentration contributes significantly to the variability of the pharmacokinetic tacrolimus parameters dnC_0, $dnAUC_{0\text{-}12}$ and dnC_{max} while in the late posttransplant recipient group the CYP3A5 A6986G polymorphism and the body mass index (BMI) have an important influence on the variability of different pharmacokinetic tacrolimus parameters. Previously, Haufroid *et al.*[12] reported a significant contribution of the CYP3A5 A6986G polymorphism and the time since transplantation on the variation of tacrolimus C_0 concentrations and the tacrolimus dose requirement. Additionally, we have also demonstrated in a stable Chinese renal transplant recipient population that the CYP3A5 A6986G polymorphism is the most significant independent variable when considering the daily tacrolimus dose as a dependent variable[30]. Kuypers *et al.*[32] found higher tacrolimus peak concentrations for female renal transplant recipients compared to their male counterparts and a significantly higher tacrolimus $AUC_{0\text{-}12}$ in female recipients six months after transplantation. Furthermore, Kuypers *et al.*[32] found lower tacrolimus trough concentrations with increasing age. In the present study, a significantly higher allele frequency of the CYP3A4*1B and CYP3A5*1 allele is observed in the early renal transplant recipients compared to the late renal transplant recipients. The higher allele frequency for the CYP3A4*1B variant allele and CYP3A5*1 allele in the early renal transplant recipient group may partly clarify the difficulties in achieving and maintaining an optimal tacrolimus blood concentration. Although earlier studies[20-22] reported a weak significant association between ABCB1 genotypes or haplotypes with tacrolimus C_0 concentrations of the transplant patients, no differences were found between either the different ABCB1 polymorphisms or the ABCB1 haplotypes and the

pharmacokinetic tacrolimus parameters in the two renal transplant recipient groups. Recently, Fredericks *et al.*[33] reported after examining 206 stable renal transplant recipients that the individual ABCB1 polymorphisms and ABCB1 haplotypes show a relatively minor association with the tacrolimus pharmacokinetics which implies that genotyping for ABCB1 polymorphisms seems to be of minor importance for predicting the daily tacrolimus dose regime. CYP3A enzymes are responsible for the most important metabolic route of tacrolimus, namely its demethylation to 13-O-demethyltacrolimus[34-37]. Due to the lack of CYP3A5 activity caused by the CYP3A5*3 variant allele, transplant patients who were homozygous for the CYP3A5*3 variant allele required an almost twofold lower daily tacrolimus dose and achieved even a higher dnC_0, $dnAUC_{0\text{-}12}$ and dnC_{max} compared to the transplant recipients that carry at least one CYP3A5*1 allele. Although in the present study a trend is observed between the different CYP3A4 A-392G genotypes and the pharmacokinetic tacrolimus parameters, the contribution of this CYP3A4*1B variant allele seemed limited. After selecting two renal transplant recipient groups, one group with the genotype combination CYP3A4 *1A/*1A – CYP3A5 *1/*3 and another group with the genotype combination CYP3A4 *1A/*1B – CYP3A5 *1/*3, no significant differences were observed between these groups and the daily tacrolimus dose as well as the dnC_0, $dnAUC_{0\text{-}12}$ and dnC_{max}. This may indicate that the influence of the CYP3A4 *1B variant allele is restricted and that at least most of the genetic effect is being caused by the CYP3A5 A6986G polymorphism. These data seem to conflict with a previous study in which Hesselink *et al.* reported that the CYP3A4*1B variant allele has a significant influence on the daily tacrolimus dose despite they observed a 80% overlap between the CYP3A4*1B and CYP3A5*1 allele[15]. Although tacrolimus is a substrate of CYP3A4, our findings suggest that if there is an influence of this CYP3A4*1B polymorphism, it is probably caused by the high linkage between CYP3A4*1B and CYP3A5*1. Summarised, it appears that carriers of CYP3A5*1 allele included, in either the early or the late postransplant recipient group, show a twofold lower dnC_0, $dnAUC_{0\text{-}12}$ and dnC_{max} for tacrolimus compared to homozygous carriers of a CYP3A5*3 variant allele. Thus carriers of a CYP3A5*1 allele require a twofold higher tacrolimus dose compared to homozygous carriers of a CYP3A5*3 variant allele. Therefore, we conclude that genotyping for the CYP3A5*3 variant allele is of great value to determine the initial and maintenance oral tacrolimus dose. By doing so the risk of under- or over-immunosuppression in individual renal transplant recipients will be minimized.

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